# A MODEL FOR PREDICTION OF PROBABILITY OF DEVELOPING AN ADVERSE PHYSIOLOGICAL SYMPTOM IN INDIVIDUALS PERCUTANEOUSLY EXPOSED TO VX NERVE AGENT

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## ABSTRACT

A model is developed for the probability of toxic effects resulting from either vapour or liquid acute exposure of the skin to the organophosphonate nerve agent VX (O-ethyl S-2-diisopropylaminoethylmethyl phosphonothiolate), for which considerable relevant human toxicity information is available. This model focuses solely on percutaneous toxicity and incorporates population variability of response and variable permeability of the different regions of the body to the agent. An ECt50 value for nausea and vomiting due to whole body vapour VX exposure (neglecting respiratory contribution) is derived and compared with other estimated values. In addition, the model predicts the ED50 for liquid VX applied to various body regions, with a variation of about a factor of 300 predicted between the least and most sensitive body regions for which data are available. The predictions of the model appear consistent with available human effects data, and thus the effective dose predictions of the model are to be preferred over the somewhat less conservative estimates in the literature.

Keywords: Risk, chemical exposure, protective clothing, skin permeability, percutaneous, organophosphonate, nerve agent, VX

## INTRODUCTION

In work environments where toxic chemicals are used, manufactured or stored, the adoption of protective measures to minimise percutaneous exposure to hazardous chemical vapours has become an increasingly important issue for personnel that must handle these chemicals (Mattie et al., 1994). This risk is particularly noteworthy for individuals who may be acutely exposed to such lethal systemically acting chemical agents as organophosphate nerve agents (examples are sarin and VX). These individuals could include first responders to the scene of a chemical agent attack, or military personnel in the theatre of war. Thus a chemical liquid and vapour exposure risk assessment model for individuals wearing a chemical protective ensemble (CPE) that predicts the probability of developing a defined

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physiological symptom of toxicity as a result of percutaneous absorption of a toxic chemical is needed.

We have proceeded to develop such a model for the organophosphonate nerve agent VX (O-ethyl S-2-diisopropylaminoethylmethyl phosphonothiolate), for which considerable relevant human toxicity information is available. This model focuses solely on percutaneous toxicity, assuming that available respiratory protection (such as self-contained breathing apparatus and negative pressure active carbon filtered respirators) will be sufficient to avoid ill effects. One previous study has attempted to predict the likelihood of toxicity resulting from VX exposure using parathion as a simulant for VX and obtaining in vitro data on cadaver skin from a single body region (Wester et al., 2000). The model described here incorporates all the available human data in order to more accurately predict acute effects.

Specifically, there are several different types of information that must be known in order to assess the likelihood of a toxic response to an agent exposure. Firstly, the actual agent challenge received to the skin must be known, which is a relatively simple parameter in the case of the unprotected individual but much more complex in the case of the protected individual. Secondly, the rate at which the skin absorbs the agent and effectively delivers it into the bloodstream must be characterized. Finally, the likelihood of a toxic response given the actual amount of agent in the bloodstream (for a systemically acting agent such as VX) must be known.

Recently, a standardized methodology for determination of protection factor distributions has been developed in a multinational collaboration, which permits determination of this information for any CPE (Duncan et al., 1998, Duncan and Gudgin Dickson, 2001, Chemical Biological Defence Technical Panel 11, 1997, Duncan and Gudgin Dickson, 2003). Thus we can predict for the population exposed under a given set of conditions what the vapour challenge to each body region is likely to be.

It is well established that the body itself has greatly variable permeability coefficients over various body regions (Stoughton, 1989). Permeability coefficients vary with the ambient temperature, the individual's activity level (Danon et al., 1986), and the specific physical characteristics of the skin of an exposed individual, such as variability in epidermal thickness and density (Rins et al. 1991), the integrity of the stratum corneum (Benfeldt et al. 1999), the presence of ducts and glands (Kao et al. 1988), follicle area opening (Scott et al. 1991), hydration (Wurster et al., 1979, Idson, 1978), skin temperature (Danon et al., 1986, Craig et al., 1977), subdermal fat deposits and cutaneous vasculature and blood flow (Danon et al., 1986, Monteiro-Riviere et al. 1993, Pershing et al., 1989), and possibly other factors. The large variation in cutaneous absorption rates is rarely addressed in the measurement of skin permeation of chemicals; often absorption rates are measured at a single site such as the forearm. Systematic reports of percutaneous liquid absorption rates and regional variations in sensitivity to liquid chemical exposures are available in the literature (Craig et al., 1977, Maibach et al. 1971, Feldmann and Maibach, 1967, Sim, 1962, Heinen et al., 1945, Berner and Cooper, 1987, Blank, 1964, Blank and McAuliffe, 1985, Fredriksson, 1962, Hodge and Steiner, 1943, Jacobs and Phanprasit, 1993, Michaels et al., 1975, Riihamäki and Pfäffli, 1978, Roberts and Anderson, 1975, Scheuplein et al., 1969, Treherne, 1956, Valette et al., 1954, Wester and Maibach, 2002). Of particular interest to this work are those reports (some of which are not available in the open literature) pertaining to the nerve agent VX, for which a significant route of exposure would be dermal uptake (Sim, 1962, Sim and Stubbs, 1960, Duncan et al., 2002). With knowledge of how the liquid permeability rates vary over the

body we can predict the likely amount of VX reaching the bloodstream if liquid is applied to various body regions either uniformly or nonuniformly. A complete set of regional vapour percutaneous permeability coefficients for VX does not exist, nor, to our knowledge, does it exist for any compound in the open literature. In the case of VX, we have used the liquid permeability rates to predict the vapour permeability coefficients for corresponding body regions. Given this information, we can predict the distribution in the dose of VX that would reach the bloodstream under a given set of exposure conditions (either liquid or vapour) for the protected or unprotected population.

Finally, we must predict the likelihood of a toxic reaction occurring as a result of the dose of VX received in the bloodstream. There are human toxicity data for VX that permit correlation of the VX dose administered intravenously and the likelihood of the severe incapacitating physiological effects (nausea/vomiting). With this dose-response information incorporated into the model, we can predict the likelihood of such effects in the protected or unprotected population exposed to a VX liquid or vapour challenge.

The model is actually developed here in the reverse order. Firstly, knowledge of the correlation between a measurable VX dose parameter and the desired toxic symptom is required. Secondly, the likelihood of receiving such a dose via percutaneous absorption of either liquid or vapour in the unprotected population for a given scenario is examined. These two steps are addressed in the current study and the results compared with existing human toxicity data and estimates. Finally, the effect of wearing a CPE on the likelihood of receiving such a dose must be added into the model, as a subject of future work. At each step, there is a population distribution that must be determined and incorporated into the calculation in order to determine probability of toxic effects.

## DEVELOPMENT OF THE MODEL

### *Relationship between VX dose and toxic symptoms*

We are first interested in knowing the dose-response relationship between some measurable systemic dose of VX and a specific incapacitating physiological reaction. Sidell (1997) has reviewed a number of published and unpublished studies on VX administration in humans having received VX by various routes. A pronounced relationship between the resulting depression of red blood cell cholinesterase (RBC ChE) activity and the physiological symptom of vomiting after VX exposure was observed. These data have been summarized in Figure 1.

If we can now develop a relationship between a VX dose delivered intravenously and RBC ChE activity, we can ultimately correlate a measurable VX dose with the specific toxic response of vomiting. Kimura et al. (1960) documented the depression in the red blood cell cholinesterase (RBC ChE) activity in humans resulting from an intravenous infusion of VX into the blood. One subject was given an intravenous infusion up to 2.1 micrograms per kilogram over 5.5 hours, while 4 subjects were given an intravenous infusion up to 1 micrograms per kilogram over 4 hours and 2 over 2 hours. These data are summarized in Figure 2. The empirical fit to the data is described by a semi-logarithmic relationship

$$\ln R_A = -0.95 m_B^{1.1} \qquad [1]$$

where RA is the RBC ChE activity as a fraction of control, and mB is the dose of chemical in the blood micrograms per kilogram. The inverse of Equation [1] gives the fitted relationship between the intravenous dose and the RBC ChE activity:

$$m_B = \left( \frac{\ln R_A}{-0.95} \right)^{0.91} \qquad [2]$$

We can combine these two sets of data in order to obtain a classic "S-shaped" dose-response relationship relating the physiological symptom of vomiting to the dose of chemical in the blood, illustrated in Figure 3. The functional form of the fitted curve is that of a log-normal cumulative probability distribution

$$P_D(m') = \int_0^{m'} D(m_B, \mu_D, \sigma_D) dm_B \qquad [3]$$

where mB is the dose of chemical in the blood, m varies between the limits $0 \le m' \le \infty$, and D(mB,μD,σD) is a log-normal probability density function:

$$D(m_B, \mu_D, \sigma_D) = \frac{1}{m_B \ln(\sigma_D)\sqrt{2\pi}} \exp\left[ -\frac{1}{2} \left( \frac{\ln m_B - \ln(\mu_D)}{\ln(\sigma_D)} \right)^2 \right] \qquad [4]$$

in which μD is the geometric mean and σD is the geometric standard deviation of the distribution of the intravenous dose of chemical mB. The geometric mean and geometric standard deviation for the fitted D(mB,μD,σD) have been determined from a normal equivalent deviate (NED) dose-response curve constructed from the data in Table 11. The value obtained for the geometric mean is 1.45 and for the geometric standard deviation 1.4. We now can predict the likelihood of vomiting for the population (as represented by the 19-34 year old males in the study) having received a given intravenous dose of VX.

### *Calculation of the Systemic Mass Absorbed for the Exposure of an Unprotected Individual to VX Liquid or Vapour*

*Regional liquid VX permeability*

There is available a study performed by Sim (1962) in which the variability of different sites on the body to percutaneous penetration of liquid VX was examined. Here, we wish to use Sim's data in order to infer skin permeabilities of various body regions to liquid and vapour VX. Skin permeability has been shown to vary for certain chemicals over different regions of the body, and Sim's study confirmed this observation for liquid VX.

A topical dose of neat liquid VX was placed (as one or more drops) on the skin of volunteers at different regions of the body, at 24 degrees Celsius, 60 percent RH, using 4 to 14 individuals per body region (typically 8). The VX was left in situ for a period of 24 hours, during which time samples of blood were drawn from the individual and the RBC ChE activity measured.

We wish to calculate from these data the liquid penetration rate, or flux as it is sometimes called, as the amount of chemical that has penetrated the skin and entered the systemic circulation per unit area, per unit time:

$$F_L = \frac{m_B w}{tA} = \frac{m_T}{tA} \qquad [5]$$

where, in the case of liquid VX exposure, FL is the penetration rate of the liquid in micrograms per centimetre squared per minute, mB is the intravenous dose of VX in micrograms per kilogram, w is the subject's weight in kilograms (with the product of these two parameters being mT the total amount of VX in the blood), t is the time to minimum RBC ChE activity in minutes, and A is the surface area, in centimetres squared, exposed to the liquid. The magnitude of FL will vary between body regions, and for the population will be represented by a log-normal distribution.

*Model assumptions*

In order to use the available data to calculate the liquid penetration rate, the following assumptions are made:

(i) the rate-limiting step for this entire process is the penetration of the agent through the skin and the subsequent time to cause inhibition of RBC ChE activity, once the agent is in the bloodstream, is small compared to the diffusion time through the stratum corneum,
(ii) the area of the droplet as measured at time t of minimum cholinesterase activity is representative of its area over the entire absorption process and directly proportional to the amount penetrated (that is, a larger surface area drop would result in more penetration over the same length of time),
(iii) free cholinesterase generation during time t is negligible, and
(iv) the rate of agent diffusion through the skin barrier and subsequent inhibition of ChE is approximately constant over the entire time until the minimum is reached.

The assumptions above are discussed further here. Regarding assumption (i), that penetration through the skin is the rate limiting step, from the available information it is clear that the stratum corneum acts like a passive (but not necessarily an inert) diffusion medium. The large amount of in vitro research on skin permeability and solid experimental data testifies to the widespread acceptance of this view (Ainsworth, 1960, Berenson and Burch, 1951, Blank, 1964, Tregear, 1966). The permeability of water, for example, is 0.2-0.4 milligrams per centimetre cubed hours at 30 degrees Celsius whether measured in vivo from a nonsweating region of the forearm or abdomen (Baker and Kligman, 1967), or in vitro from excised skin (Baker and Kligman, 1967, Burch and Winsor, 1946). Similar correlations between in vivo and in vitro experiments have been observed for ions (Sprott, 1965) and for nonelectrolytes also (Scheuplein et al., 1969). It has also been concluded that passive diffusion through the stratum corneum is the major route of skin penetration for the related organophosphonate nerve agent, sarin (Wurster et al. 1979). Assumption (i) is thus presumed to be valid.

The amount of error introduced by assumption (ii), constant skin area covered by the liquid VX, is not clear. If the area of the VX droplet was not reasonably constant during the time to minimum RBC ChE activity, the calculated permeability rate could be over- or underestimated. In fact, Sim does give the initial droplet area compared with the maximum spread used in the model calculations, and in a few cases this value has increased by as much as 100 percent; in general the change was perhaps 10-20 percent. It cannot be told from the data how long it took for the maximum spread to be reached, i.e. over what fraction of the time of application the drop was actually covering the maximal surface area used.

Regarding assumptions (iii) and (iv), no regeneration of free ChE and a constant diffusion rate through the skin, Sim's data demonstrated that ChE activity dropped in an approximately linear manner until it reached a minimum, then remained constant within error between the time of maximum inhibition and 24 hours after application of the VX, although recovery did occur subsequently over the next 3-5 days (Sim, 1962). Thus assumption (iii) is valid. Assumption (iv) implies in part that there are large reservoirs of liquid VX on the surface that are not significantly depleted at any time. In a separate study by Sim and Stubbs (1960) on application of liquid VX to the forearm, under similar conditions to those in Sim, a maximum of 6 percent of the applied dose was absorbed at doses in the range of 5-35 micrograms per kilogram. In Sim's study, somewhat larger fractional doses may have been achieved in the more sensitive areas (based on the IV dose calculated from the observed RBC ChE activities). The highest geometric mean fractional dose absorbed was estimated at about 30 percent for the ear, but was less than 10 percent for most other body regions.

*Calculation of liquid penetration rates*

Using the relationship given by Equation [1] as fitted to Figure 2, and the RBC ChE activity data in Sim (1962), we have determined the systemic level of chemical in the blood that should have given rise to the observed RBC ChE activity. Knowing the blood dose, the assumed weight of the subject, the surface area covered by the VX drop and the time to minimum RBC ChE activity, we have then determined for each subject the percutaneous penetration rate for liquid VX for the given body region using Equation [5].

An example of the data obtained for the cheek by Sim is provided in Table 2. A dose of 5 micrograms per kilogram VX was applied to the cheek of 8 subjects. The subject identification code, subject weight, initial surface area covered by the droplet, and maximum surface area are listed in the first 4 columns; the topical VX dose, time to minimum RBC activity and the minimum RBC ChE activity level measured in each subject are given in columns 5, 6 and 7.

Table 2 also demonstrates the calculation of percutaneous penetration rates for the cheek. Table 3 summarizes the liquid penetration rate results calculated in this manner for all of the body regions studied by Sim.

*Calculation of vapour permeability coefficients*

In the absence of detailed vapour exposure data for VX over all body regions, the liquid penetration rates will next be used in order to infer percutaneous VX vapour permeability coefficients.

The penetration rate for vapour Fv may be defined in terms of a concentration of chemical in the vapour phase C in micrograms centimetres cubed and a proportionality rate constant (the permeability coefficient $\nu$, in centimetres per minute):

$$F_\nu = C\nu \qquad [6]$$

In order to be able to use the values of FL to calculate ν for each body region, we must be able to relate FL to Fv. To do this, we assume that C in the liquid case corresponds to the volatility of the liquid under the conditions of the exposure (the vapour concentration in the saturated state). We also assume that this saturated vapour concentration is a reservoir of chemical that is large in comparison with the skin's total capacity for absorption (as in the liquid case). Further, it is assumed that the stratum corneum very rapidly approaches equilibrium with the saturated vapour above it. In effect, the rate-limiting step is considered to be the transport of the agent through the complete epidermal/dermal layer and not the dissolution of the agent into the stratum corneum. The implication of these assumptions is that, regardless of whether the VX was delivered to the stratum corneum via a liquid or a saturated vapour medium, the mass of VX reaching the microcirculation layer and ultimately entering the blood would be equivalent. This assumption is thermodynamically sound but other factors such as kinetics or physiology could affect its validity; for example, application of a liquid to the skin may affect its barrier properties over a long period in a manner different from that of the saturated vapour.

In detail, equating FL to Fv and setting C in Equation [6] equal to the volatility or saturation concentration of VX vapour Cs, we have

$$\nu = \frac{F_L}{C_S} \qquad [7]$$

where ν is the VX vapour percutaneous permeability coefficient expressed as a function of the liquid penetration rate FL and the saturation vapour concentration Cs of VX. The saturated vapour concentration Cs (volatility) of VX at a temperature of 32 degrees Celsius, which is a representative temperature for the skin, is estimated as 0.0191 micrograms per centimetre cubed (calculated from the Clausius-Clapeyron equation using the following values: volatility at 25 degrees Celsius 0.0105 micrograms per centimetre cubed; boiling point 298 degrees Celsius; and molecular weight 267 grams per mol (Compton, 1988).
Table 3 lists the geometric mean calculated percutaneous permeability coefficients and their associated geometric standard deviations for all of the body regions investigated by Sim (1962).

*Further requirements for the model*

If we wish to use the permeability coefficient (and penetration rate) values presented in Table 3 in a general way, for realistic exposure scenarios, we must make the following further assumptions:

(i) human skin temperature is a constant 32 degrees Celsius, hence, the variation of saturated VX vapour pressure with temperature is not incorporated;
(ii) the penetration rates are independent of temperature and relative humidity;
(iii) the permeability coefficient for each body region is independent of the vapour concentration; and

(iv) the distributions of penetration rates obtained (Sim, 1962) are representative of the population of interest (no gender, skin type, age, size bias).

All of these simplifying assumptions can clearly be in error in certain cases. For example, increased temperature, humidity and activity level all can increase rates of penetration for liquids perhaps primarily due to increase in hydration of the stratum corneum reducing the diffusion barrier (Danon et al. 1986, Craig et al., 1977); for liquid VX an increase from 18 to 46 degrees Celsius increased permeability by about a factor of 3 for the cheek and forearm (Craig et al., 1977). Skin temperature is not expected to vary to such an extent for the individual wearing protective clothing, which will impose a heat burden resulting in relatively high skin temperatures over the entire body. Finally, there is definite population bias due to use of 18-34 year old healthy males in the military for the original study; this bias is not as severe as it might have been, as we wish to extrapolate to physically fit first responders and military personnel typical of the study group, nevertheless gender and skin type profiles were not representative of this population.

In addition, in order to use these values in general for whole-body exposures, we must have a reasonably complete set of body regions represented. One noteworthy absence from Sim's investigation was the region of the scrotum. Studies by Maibach et al. on cortisol (Feldmann and Maibach, 1967) and parathion uptake (Maibach et al., 1971) suggest that this body region is especially significant, exhibiting notably higher penetration rates than other areas of the body. In both studies, the ratio of the scrotum value to the jaw values is about 3. It has been noted that parathion should be a good simulant for VX due to its similar functional groups and molecular weight (Wester et al., 2000). Applying this same ratio to the VX data yields a scrotum penetration rate of 0.16 milligrams per centimetre squared minutes for VX liquid and a permeability coefficient of 8.3 centimetres per minute for VX vapour. This is slightly greater than the next highest VX permeability coefficient determined at the ears. Again there is an obvious potential gender bias associated with this particular regional value.

Finally, in order to have appropriate coverage of the entire body at regions critical to performance for the individual wearing chemical protective equipment, we have generated skin permeability coefficients and liquid penetration rates at a number of additional body regions. Data of Table 3 were mapped to other body regions by averaging values from various nearby sites or splitting or combining body regions as originally given in Sim (1962). These values are given in Table 4. It is the geometric mean values that are subsequently to be used to calculate predicted IV blood doses for individuals either without protection, or wearing CPE and exposed to a VX challenge. The individuals are assumed to have a standard body size for simplicity, with the assumption that corrections to body size could be superimposed subsequently and would roughly scale over the entire body for a given individual. The variability in the uptake of chemicals amongst the population should also be taken into consideration. This is done by initially assuming that the same difference that is observed in rate of penetration for a particular body region between two individuals would apply to all other body regions. In other words, an individual that is more sensitive than average at one region is more sensitive at all other body regions by the same factor. Thus the average of the geometric standard deviations for the all body regions, a value of 2.1, is used to express the population variability of cutaneous absorption. This population variability can then simply be expressed as the variability that would ultimately be observed in the intravenous dose received.

The probability of effect PEFF is the integral of the interaction between D (as given by equation 3) and PD (as given by equation 2) over all intravenous doses m, as follows:

$$P_{Eff}\left(\mu_{m_P},\sigma_{m_P},\mu_D,\sigma_D\right)=\int_0^\infty D(m,\mu_{m_P},\sigma_{m_P})P_D(m)dm \qquad [8]$$

## TESTS OF VALIDITY AND COMPARISON WITH PUBLISHED VALUES

Bramwell et al. (1963) performed experiments on human volunteers in which the entire head and neck area was exposed to VX vapour, with respiratory protection provided by a breathing tube in the mouth, for Ct's in the range of 0-25 milligram minutes per meter cubed and times of exposure in the range of 1.5 to 7 minutes. RBC ChE activity was measured at intervals after administration of the vapour and the activity at maximum depression was noted. This maximum depression occurred at times in the range of 4-22 hours after receipt of the vapour exposure. We have used the model developed here to calculate the expected IV dose received for each of the volunteers in Bramwell's experiments, assuming each had geometric mean permeability coefficients over the head and neck region. A plot of the observed RBC ChE activity as a function of predicted IV dose is given in Figure 4, along with the data of Kimura et al. that were used to develop the model. It can be seen that the model predicts intravenous doses resulting from Bramwell's exposures that correlate well with the observed depression of RBC ChE. Thus the permeability coefficients calculated from Sim's liquid data for the head region, assuming that liquid behaves as does saturated vapour, combined with the regional uptake analysis, appear to be consistent with the observed data from independent vapour experiments. Adding in the prediction of severe effects based on observed RBC ChE depression, the model predicts an ECt50 for percutaneous head and neck exposure of 28 milligram minutes per meter cubed, with a probability of severe effects of 0.42 at the highest exposure Ct used of 25 milligram minutes per meter cubed. Of the 35 exposures performed in Bramwell (1963), only one individual experienced severe symptoms of nausea/vomiting, that individual having received the highest Ct of 25 milligram minutes per meter cubed, and having an RBC ChE activity of 0.3. These results are summarized in Figure 5 along with the model's predictions for severe effects over the same exposure range. It can be seen that Bramwell et al.'s observations are within the range of the model's predictions given the limited statistics (where at most one individual would be expected to have shown effects for any given Ct range in the graph).

Another study has been performed by Cresthull et al. (1963) in which healthy adult males exposed one arm to VX vapour. In this experiment, 500 centimetres squared of the forearm or 1000 centimetres squared of the entire arm (not including hand) were exposed to vapour for times in the range of 5-75 minutes and vapour dosages in the range of 6-785 milligram minutes per meter cubed. RBC ChE was measured at 2 and 20 hours after exposure (not at the time of maximum depression). Values 20 hours after exposure have been used, which based on Sim's and Bramwell's studies should be reasonably representative of the minimum value as maximal depression should have been reached with no recovery. The model has again been used to predict the intravenous doses that would have been received under these same exposure conditions for the geometric mean individual. The assigned areas of the body regions used in the model have been slightly reduced to match the actual exposure areas of the experiment. The results of this calculation are presented in Figure 6. It can be

seen that although the data for the 500 centimetres squared forearm exposure fall in line with the intravenous data used in the model, the data for the 1000 centimetres squared arm exposure at higher Ct values do not. In fact, the results obtained at higher exposures (corresponding to calculated IV doses greater than 1 microgram per kilogram) show a significant degree of scatter, as well as relatively large uncertainties on the RBC ChE activities measured for all the data (viz measured increase of 18 percent in one case). Thus, for lower Ct exposures, the results of Cresthull et al. (1963) do appear to correlate well with the model's predictions, while for the higher exposure conditions on the whole arm they do not.

In Cresthull et al.'s study, no individuals developed any severe effects. It is clear by comparing the predicted IV doses with the RBC ChE depression curve of Figure 1 and the dose-response curve of Figure 3, that for the higher exposures to the whole arm, corresponding to predicted IV doses of greater than 3 micrograms per kilogram, according to the model most or all of the individuals involved should have experienced significant RBC ChE depression and hence severe and potentially lethal effects. Thus either the permeability coefficients calculated for the whole arm within the model greatly overestimate the actual rate of uptake of the VX under these conditions, or there is some shortcoming in the data of this report. If the latter is true, the most consistent explanation is that a much lower vapour dosage was actually delivered than was reported for the whole arm exposures. If we only examine the likelihood of severe effects having been observed based on the relationship of Figure 1, only one individual (that individual with a 0.24 RBC ChE activity) had any significant chance of having experienced a severe effect, about a 50 percent likelihood. Thus the results of Cresthull's study are at least consistent with that portion of the model data.

The consistency of the results obtained from this model with currently accepted toxicity predictions for VX can be tested by comparing with published estimated ECt50 values for vomiting resulting from whole-body VX vapour exposure. Both the Subcommittee on Toxicology Values (1997) and NATO (1997, 2003) have recommended 25-30 milligram minutes per meter cubed for severe incapacitating effects such as vomiting, based largely on data of Bramwell et al. This value has been given low to moderate confidence by the Subcommittee on Toxicology Values. NATO (1997) also suggests an ECt90 value of 41 milligram minutes per meter cubed. Both of these references have recommended corresponding ECt50 values for VX inhalation of 10 and 25 milligram minutes per meter cubed respectively. Using the model as developed here, taking into account regional variability in response, we would predict an ECt50 value of about 10 milligram minutes per meter cubed for exposure of the entire body to VX vapour, excluding any respiratory contribution, and an ECt90 value of 25 milligram minutes per meter cubed. Thus the values of the model give a conservative prediction compared with currently accepted values estimated from the same sources and from animal data.

This model should be closest to correct when representing a population of relatively fit adult males, as these are the individuals best represented by the measured permeability coefficients and the body areas used. Since we are primarily concerned with applying this model to the military and to members of the first responder community, the main data bias would appear to be due to the lack of females in the data used to generate the model data.

## LIQUID HAZARD MODEL, VERIFICATION AND COMPARISON WITH EXISTING ESTIMATES

Although this model was originally designed for assessment of vapour hazard, it can be adapted for use with liquid exposure. For example, the available information can be used while assuming a given area of coverage by liquid VX in any particular body region(s) in order to predict the likelihood of effects. In this case, we use the FL values calculated for the liquid, and calculate a drop spread per unit mass for each body region from the data of Sim (1962). Average drop spreads in the range of 1-20 centimetres squared were obtained, varying by body region, for drop sizes in the range of 0.5-2 milligrams. The drop spread per unit mass and FL values used in the model are given in Table 5. By assuming a certain mass received per unit area and an exposure time of about 8-12 hours (the approximate time to maximum cholinesterase depression), we can, for a total liquid dose applied uniformly over the body, calculate the equivalent liquid area coverage in milligrams per metre squared, and the likelihood of severe effects. Using these assumptions, we obtain a surface area coverage of about 0.8-1.2 milligrams per metre squared, or an ED50 for severe effects for liquid VX of 1.8-2.7 milligrams per 70 kilogram man, compared with estimates of 5 milligrams per man from NATO (1997) and a lowered recent estimate of 2.5 milligrams per 70 kilogram man (with an indication that perhaps it should be lowered still further) from Reutter and Wade (1994). Thus the model's predictions for liquid effects are relatively consistent with current estimates, which are based on both less detailed analysis of the existing data and animal studies. It should be noted however that for this calculation, the model assumes an equal distribution of the liquid over the entire body surface area. If we instead assume a single drop applied to the least or most sensitive body areas, we get an ED50 range of 30 milligrams per 70 kilogram man for the hand (dorsum, single drop) and 0.1 milligram per 70 kilogram man for the scrotum, with values lying in between for other body regions. The implications of this model are significant, as requirements for protection must take into account such body region variations. The next step is the application of the model in the case of the protected individual.

We can also perform effects predictions based on data of reference Sim and Stubbs (1960) in which single or multiple (5) drops of VX were placed on the forearm (extensor). Figure 7 shows the predictions compared with observation. Again, the observed incidence of severe effects of nausea/vomiting is consistent with the model's predictions, within the limited statistics available.

## CONCLUSIONS

Until such time as more data are available upon which to judge the model, its reasonable consistency with existing data on effects resulting from human VX exposure to both liquid and vapour forms would suggest that the more conservative ECt50 values predicted should be considered when assessing the risk resulting from percutaneous exposure to vapour or liquid VX.

The next step involves taking the model developed here and applying it to the case of a group of individuals wearing chemical protective ensembles exposed to a VX vapour, where variable vapour protection is received over the various body regions.

## FOOTNOTES

[1]The dose at P=0.5 corresponds to the geometric mean at NED=0, and the geometric standard deviation is determined from the slope of the NED plot:

$$\sigma_D = 10^{1/b},$$

where b is the slope.

## TABLES

### Table 1. Intravenous dose associated with RBC ChE activity and the probability of vomiting

| Number of Individuals | Individuals Vomiting | Probability of Vomiting | *RBC ChE Activity (fraction of control) | Intravenous Dose (μg/kg) |
|---|---|---|---|---|
| 166 | 1 | 0.006 | 0.545 | 0.63 |
| 24 | 2 | 0.083 | 0.445 | 0.86 |
| 27 | 9 | 0.333 | 0.345 | 1.10 |
| 42 | 19 | 0.452 | 0.245 | 1.40 |
| 24 | 16 | 0.667 | 0.145 | 1.85 |

*median values for Sidell (1997) bin ranges

### Table 2. Percutaneous penetration rates for liquid VX on cheek, (24 °C, 60% RH), calculated from example data from Sim (1962): application of 5 μg/kg liquid VX to cheek.

| Subject ID | Body Weight w (kg) | Surface Area of VX Drop A (cm2) Initial | Surface Area of VX Drop A (cm2) Max | Topical Dose of VX (μg) | Time to min RBC ChE Activity t (h) | Min RBC ChE Activity (fraction of control) | Systemic Blood Dose mB (μg/kg) | Subject weight (kg) | Total Systemic Blood Dose (μg) | Topical Dose of VX applied (μg) | Fraction VX absorbed into blood | Penetration Rate FL (μg/(min. cm2)) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 220-60 | 76 | 1.30 | 3.80 | 380 | 10 | 0.38 | 1.02 | 76 | 77 | 380 | 0.20 | 0.034 |
| 221-60 | 88 | 1.30 | 3.00 | 440 | 10 | 0.32 | 1.18 | 88 | 104 | 440 | 0.24 | 0.058 |
| 222-60 | 64 | 1.50 | 1.80 | 320 | 8 | 0.26 | 1.37 | 64 | 88 | 320 | 0.27 | 0.102 |
| 223-60 | 88 | 1.20 | 2.50 | 440 | 4 | 0.31 | 1.21 | 88 | 106 | 440 | 0.24 | 0.177 |
| 228-60 | 77 | 1.50 | 6.60 | 385 | 6 | 0.50 | 0.75 | 77 | 58 | 385 | 0.15 | 0.024 |
| 238-60 | 88 | 1.00 | 3.00 | 440 | 10 | 0.23 | 1.49 | 88 | 131 | 440 | 0.30 | 0.073 |
| 232-60 | 70 | 1.80 | 4.80 | 350 | 4 | 0.28 | 1.31 | 70 | 91 | 350 | 0.26 | 0.079 |
| 233-60 | 70 | 1.50 | 2.40 | 350 | 4 | 0.05 | 2.84 | 70 | 199 | 350 | 0.57 | 0.346 |

Geo mean FL = 0.080, geo std dev = 2.

**Table 3. Calculated geometric mean and geometric standard deviation of human skin VX percutaneous penetration rates and vapour permeability coefficients (calculated in section "Calculation of vapour permeability coefficients") for different regions of the body.** In most cases values are calculated from data for a single drop, except where noted. Scrotum calculation outlined in section "Further requirements for the model"

| Body Region | Geometric Mean VX liquid penetration rate (µg.cm-2.min-1) | Geometric Mean VX vapour permeability coefficient (cm.min-1) | Geometric Standard Deviation | Number of individuals |
|---|---|---|---|---|
| Cheek | 0.080 | 4.2 | 2.2 | 8 |
| Ear | 0.12 | 6.5 | 1.9 | 6 |
| Scalp | 0.060 | 3.1 | 1.8 | 7 |
| Forehead | 0.061 | 3.2 | 1.7 | 8 |
| Groin | 0.024 | 1.2 | 1.4 | 4 |
| Nape | 0.0093 | 0.49 | 1.8 | 7 |
| Axilla | 0.046 | 0.75 | 1.7 | 7 |
| Popliteal Space | 0.010 | 0.54 | 2.8 | 9 |
| Abdomen | 0.0070 | 0.37 | 1.9 | 7 |
| Elbow | 0.018 | 0.94 | 2.3 | 5 |
| Back | 0.0034 | 0.18 | 2.8 | 8 |
| Forearm extensor | 0.0074 | 0.39 | 3.3 | 12 |
| Forearm flexor* | 0.0025 | 0.13 | 3.3 | 39 |
| Forearm flexor** | 0.0034 | 0.18 | 1.8 | 8 |
| Scrotum | 0.23 | 12 | - | |
| Buttocks | 0.0027 | 0.14 | 2.3 | 8 |
| Foot dorsum | 0.0020 | 0.10 | 2.2 | 8 |
| Foot plantar | 0.0044 | 0.23 | 1.7 | 8 |
| Knee | 0.0025 | 0.13 | 1.4 | 5 |
| Hand palmar | 0.0053 | 0.26 | 2.0 | 8 |
| Hand dorsum** | 0.0007 | 0.036 | 3.6 | 12 |

* From Sim & Stubbs forearm study; 1 or 5 drops

** 1 or 5 drops

**Table 4. Geometric mean VX vapour permeability coefficients transposed to new body regions.** The surface areas assigned to each region are also given, based on the medical "rule of nines", with further estimated subdivisions.

| Body Region for Model | Area of Body Region (A, cm2) | Geometric Mean VX vapour percutaneous permeability coefficient (ν, cm.min-1) | Body Regions Investigated by Sim (1962) used to generate values of ν |
|---|---|---|---|
| Scalp | 350 | 3.11 | Scalp |
| Ears | 50 | 6.53 | Ears |
| Face, Cheeks & Neck | 300 | 2.34 | Cheek, nape |
| Chin & Neck | 200 | 2.34 | Cheek, nape |
| Nape | 100 | 0.49 | Nape |
| Abdomen | 2858 | 0.37 | Abdomen |
| Back | 2540 | 0.17 | Back |
| Axillae | 200 | 0.75 | Axillae |
| Upper Arm medial | 488 | 0.57 | Axillae, forearm flexor |
| Upper Arm lateral | 706 | 0.33 | Nape, forearm extensor |
| Elbow fold | 50 | 0.54 | Popliteal space |
| Elbow | 50 | 0.25 | Upper arm lateral, forearm flexor |
| Forearm extensor | 487 | 0.39 | Forearm extensor |
| Forearm flexor | 706 | 0.18 | Forearm flexor |
| Hands dorsum | 200 | 0.04 | Hand dorsum |
| Hands palmar | 200 | 0.26 | Hand palmar |
| Buttocks | 953 | 0.14 | Buttocks |
| Groin | 300 | 1.25 | Groin |
| Scrotum | 200 | 8.3 | Crotch – value determined from cortisol/malathion relationship |
| Thigh anterior | 2845 | 0.34 | Popliteal space, buttocks |
| Thigh posterior | 1422 | 0.34 | Popliteal space, buttocks |
| Knee | 200 | 0.13 | Knee |
| Popliteal Space (back of knees) | 100 | 0.54 | Popliteal space |
| Shins | 1897 | 0.34 | Thigh, knee, feet dorsum |
| Calves | 948 | 0.34 | Thigh, knee, feet dorsum |
| Feet dorsum | 500 | 0.10 | Feet dorsum |
| Feet plantar | 300 | 0.23 | Feet plantar |

**Table 5. Values of liquid penetration rate and spread for body regions in model.** See Table 4 for an explanation of which body regions in Sim's 1962 study were used to map values (scrotum spread was mapped from axillae in this case)

| Body Region | FL / µg.cm-2.min-1 | Spread / cm2.µg-1 |
|---|---|---|
| Scalp | 0.0594 | 0.0032 |
| Ears | 0.1247 | 0.0055 |
| Face, Cheeks & Neck | 0.0447 | 0.0068 |
| Chin & Neck | 0.0447 | 0.0082 |
| Nape | 0.0093 | 0.0095 |
| Abdomen | 0.0070 | 0.009 |
| Back | 0.0033 | 0.0071 |
| Axillae | 0.0144 | 0.0051 |
| Upper Arm medial | 0.0109 | 0.0095 |
| Upper Arm lateral | 0.0063 | 0.0095 |
| Elbow fold | 0.0104 | 0.0074 |
| Elbow | 0.0048 | 0.0036 |
| Forearm extensor | 0.0074 | 0.0073 |
| Forearm flexor | 0.0033 | 0.01 |
| Hands dorsum | 0.0007 | 0.0066 |
| Hands palmar | 0.0049 | 0.0037 |
| Buttocks | 0.0027 | 0.011 |
| Groin | 0.0239 | 0.0098 |
| Scrotum | 0.16 | 0.0051 |
| Thigh anterior | 0.0066 | 0.0098 |
| Thigh posterior | 0.0066 | 0.0098 |
| Knee | 0.0025 | 0.0054 |
| Popliteal Space | 0.0104 | 0.0074 |
| Shins | 0.0066 | 0.0054 |
| Calves | 0.0066 | 0.0074 |
| Feet dorsum | 0.0020 | 0.011 |
| Feet plantar | 0.0044 | 0.0037 |

## FIGURES

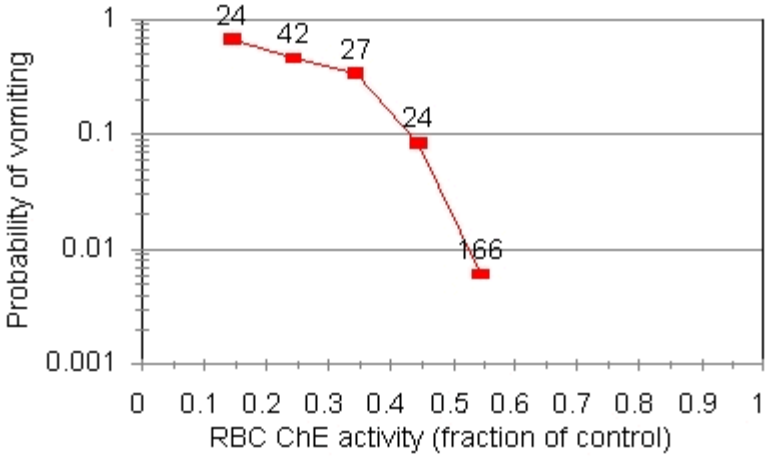


**Figure 1. Relation between RBC ChE activity and the physiological symptom of vomiting for exposure to the nerve agent VX (data from Sidell (1997)).** The sample size for each point is given above the point, the total sample size N=283

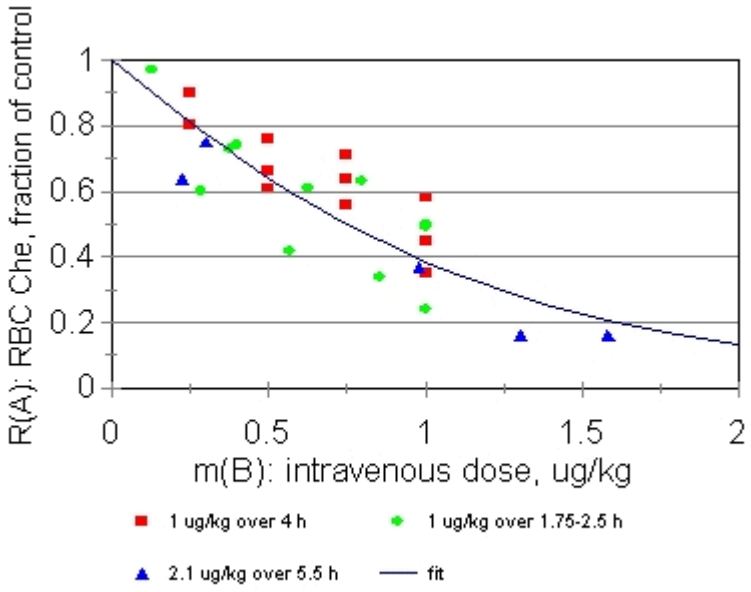


**Figure 2. The relationship between administered intravenous VX dose and resulting RBC ChE activity, based on data in Kimura et al. (1960) (N=26)**

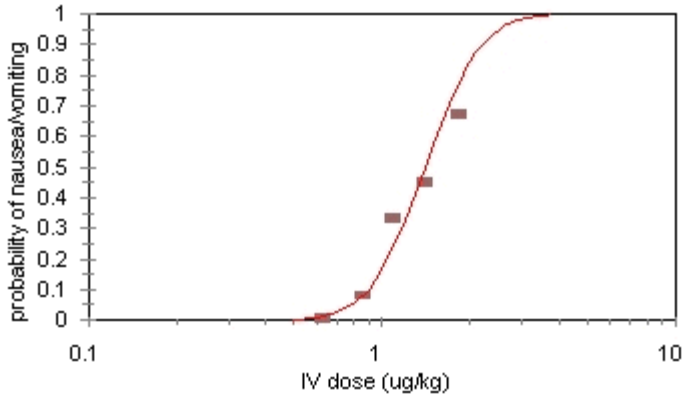


**Figure 3. Dose-response curve for vomiting as a result of administration of an intravenous dose of VX, showing data from Table 1 and fitted curve**

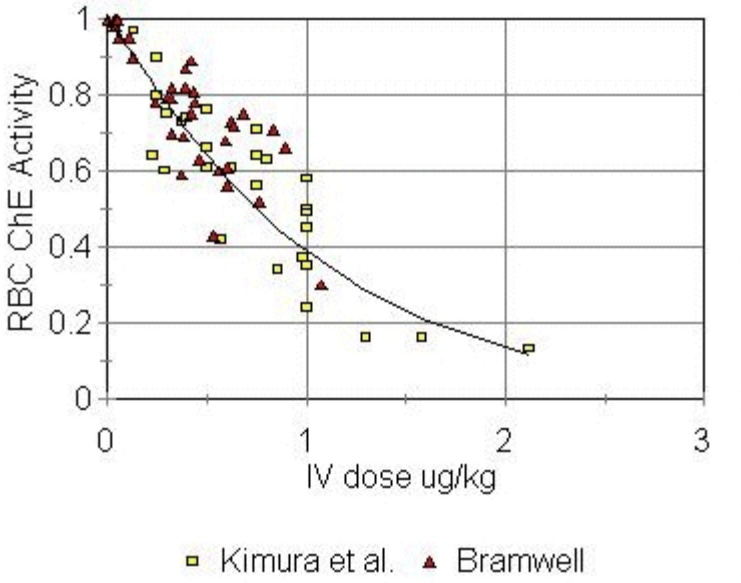


**Figure 4. Measured RBC ChE activity plotted against predicted IV dose calculated from the vapour exposure data of Bramwell et al. (1963) (triangles), compared with the intravenous data already shown in Figure 2 fitted by Equation [1] (squares/line)**

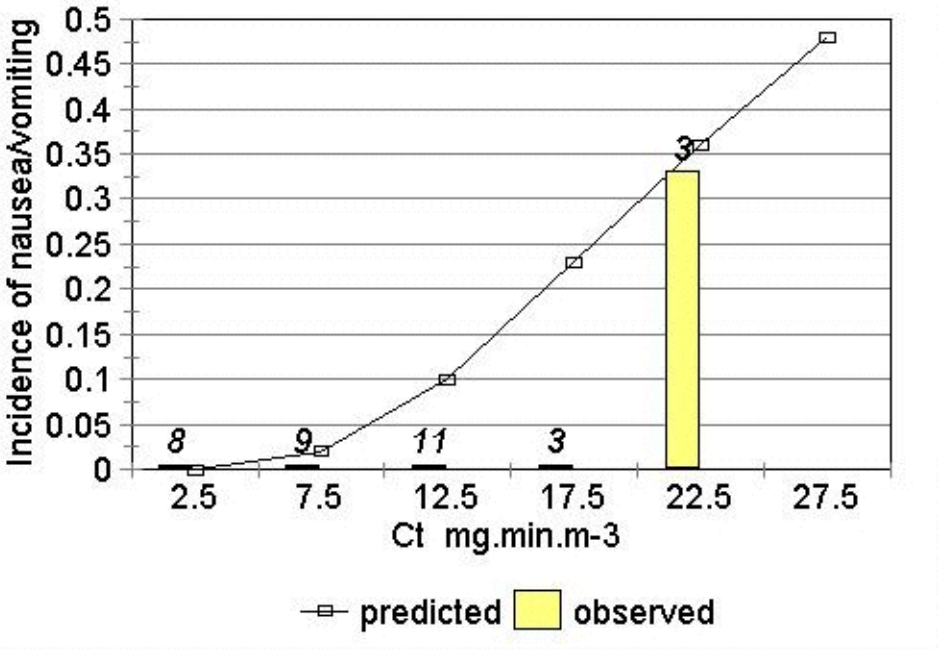


**Figure 5. A comparison between the model's predictions for incidence of severe effects compared with those observed in Bramwell et al. (1963).** (Bramwell et al. data were binned over the 5 mg.min.m-3 ranges around the Ct value on the x-axis; N, the number of observations within each bin, is given above each bar)

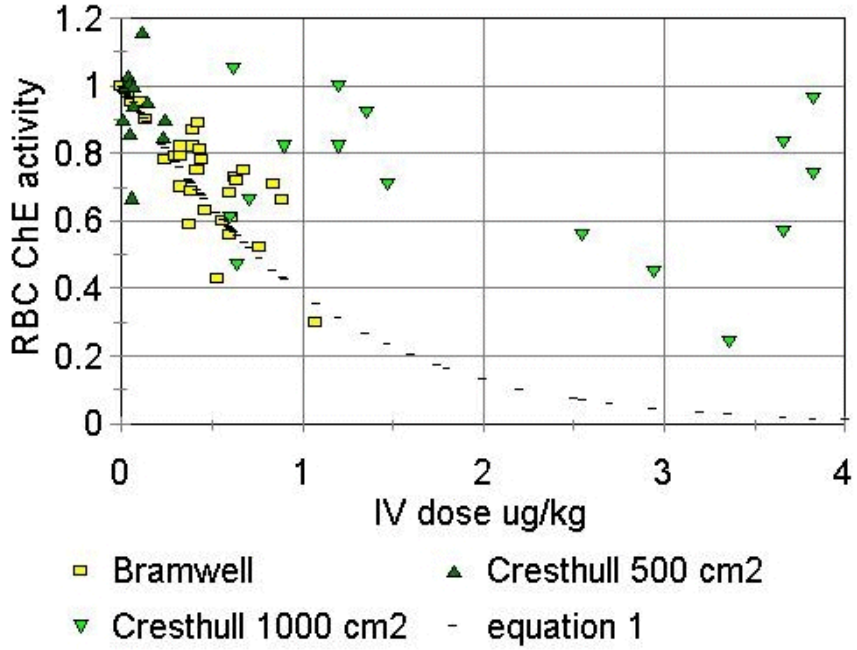


**Figure 6. The correlation between the observed RBC ChE activity and the IV dose calculated from the model for Cresthull et al.'s 1963 arm study (triangles and inverted triangles), compared with the intravenous data (squares) fitted by Equation [1]**

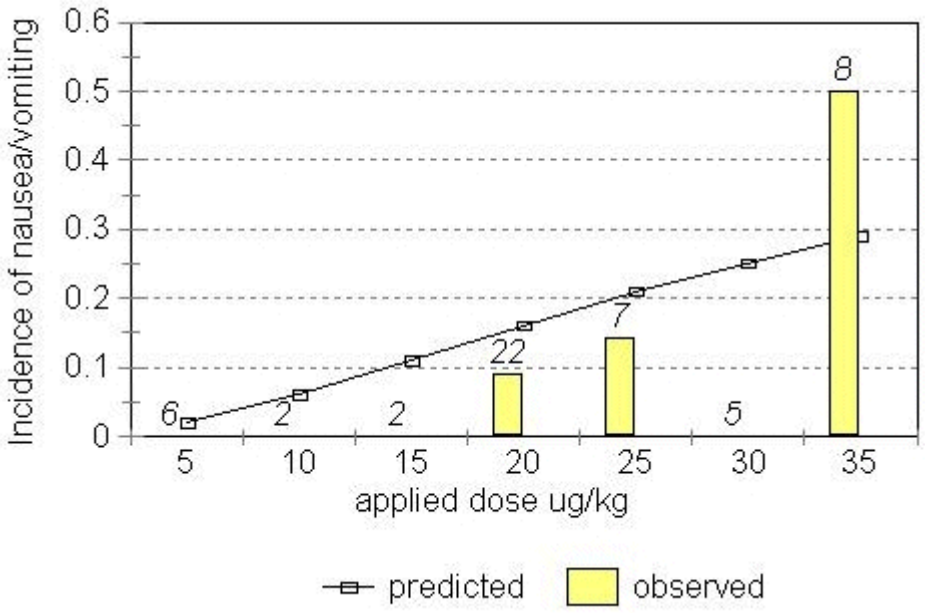


**Figure 7. A comparison between the model's predictions for incidence of severe effects from cutaneous absorption of liquid VX on the forearm compared with those observed in Sim and Stubbs (1960).** The number of observations for each applied dose is given above the bar

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